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# Polypeptide Pattern of Mesophyll and Bundle Sheath Thylakoids of Maize Chloroplasts

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**The energy converting apparatus of the photosynthesizing oxygenic species organized in several different multisubunit protein complexes is associated with thylakoid membranes. A characteristic feature of $C_4$ plants is the differentiation of the photosynthetic leaf tissues into two distinct cell types, mesophyll (M) and bundle sheath (BS) cells. In this study, polypeptide patterns of mesophyll and bundle sheath thylakoids of maize (*Zea mays* L.) have been analyzed. The amount of the PSI core apoprotein (68 kDa) was found to be higher in bundle sheath compared with mesophyll thylakoids. α and β subunits (with molecular masses of 55 kDa and 52 kDa) of CF1 domain of the ATPase complex were present in both subcellular fractions. But the amount of α subunit was smaller in the bundle sheath thylakoids. The protein of 45 kDa belonging to the core antenna of PSII was more intensive in mesophyll thylakoids. Polypeptides (with molecular masses in the region of 28-24 kDa) in the composition of the light-harvesting complex II were present in both types of thylakoids. However, in the thylakoids of bundle sheath cells their amounts were reduced.**

***Keywords:*** *$C_4$ plants, mesophyll, bundle sheath, chloroplasts, thylakoids, Zea mays*

## INTRODUCTION

In higher plants, the photosynthetic apparatus is compartmentalized in the specialized chloroplast organelle. The molecular machinery for the primary photosynthetic processes, the sunlight-driven generation of metabolic energy equivalents, is harbored in a thylakoid membrane system within the chloroplasts (Dekker and Boekema, 2005; Austin and Staehelin, 2011; Kirchhoff et al., 2013). An essential feature of the thylakoid membrane system is its high flexibility, which is required for adaptability and maintenance of the photosynthetic machinery in plants. Highly responsive to environmental conditions, the molecular membrane composition can change remarkably to optimize, protect, and maintain the photosynthetic apparatus (Melis, 1991; Walters, 2005; Jonson et al, 2011).

The protein complexes that catalyze electron transfer and energy transduction are unevenly distributed in thylakoids. The majority of the Photosystem II (PS II) complexes and light-harvesting complex II (LHC II) are largely found in the grana stacks while photosystem I (PS I) and ATP-synthase are located in the stroma exposed regions and the cytochrome $b_6$/f complex is evenly distributed in granal margins (Melis, 1991; Ke, 2001; Nelson and Yokum, 2006; Seibert, 1993; Stachelin and van-der Stay, 1996; Bukharov, Abdullayev, 1990; Andersson and Anderson, 1980; Mathis and Rutherford, 1987; Süss et al, 1993).

Photosystem II has an outer antenna dominated by light harvesting complex II (LHC II), which binds chlorophyll a, chlorophyll b, carotenoids and inner antenna of chlorophyll a binding proteins CP 47 and CP 43. The D1 and D2 polypeptides from the heterodimer of the PS II reaction center core that carries most of the cofactors are involved in electron transfer. Most proteins in the PS II complex are membrane spanning, but three extrinsic proteins involved in oxygen evolution are located on the lumenal side of the thylakoid membrane. In higher plants and green algae these proteins are nuclear encoding subunits of PsbO (33 kDa), PsbP (23 kDa) and PsbQ (16 kDa), which together form the lumenally exposed water splitting center.

A characteristic feature of $C_4$ plants is the differentiation of the photosynthetic leaf tissues into mesophyll (M) and bundle sheath (BS) cells. PS II complex is expressed in a tissue–specific manner in the NADP-ME type of $C_4$ plants (Edwards et al, 2001), predominantly in the mesophyll cells. Chloroplasts isolated from BS cells contain PS I activity, but do not photoreduce NADP from water, and cannot evolve oxygen similarly to the stroma thylakoids of $C_3$ plant chloroplasts (Lavergne and Leci, 1993). It was demonstrated that PS II in bundle sheath was inactive due to the absence of polypeptides participating in water oxidation and/or the light harvesting complex of PS II (Lu and Stemler, 2002). Moreover, it was shown that BS chloroplasts contained LHC II polypeptides but peptide

composition and amounts were different in both types of cells (Vainstein et al, 1989).

In this study, we determined the polypeptide pattern of mesophyll and bundle sheath thylakoid membranes, isolated from chloroplasts of maize.

## MATERIALS AND METHODS

A cultivar of maize (*Zea mays* L.) named Zagatala 420 was used as an object of the research. The plants were grown under the controlled condition (photoperiod -14 h light/ 10 h dark, t=26°C/14°C and the light intensity -3000 lux). It was used 28-day-old seedlings of maize. The separation of the assimilative tissues (M and BS) into subcellular fractions was done according to the method (Guliyev et al., 2003). For this purpose, the buffer solution (buffer A), which consists of 25 mM HEPES buffer (pH 7.8), 0.3 M sucrose, 1 mM EDTA – Na, 0.2% BSA and 15-20 mM 2–mertaptoethanol was used.

Chlorophyll concentration was determined spectrophotometrically in the 80% asetone extract according to the Sims and Gamon method (Sims and Gamon, 2002). The pigments were extracted from the assimilating tissues of maize (M and BS) with 80 % -acetone–Tris solution (80:20, pH 7.8), the chlorophylls a and b were spectrophotometrically measured (Ultrospec 330 Pro "Amersham", USA) at wavelengths of 663 and 647 nm in accordance with absorption spectrums.

A high-resolution gradient-electrophoresis method was developed and used in the experiments. Electrophoresis was performed in the PU-2/4LS apparatus (“Farmacia”, Sweden) using a vertical system, at 4°C, 12 mA current, 450 V, for 16 hours. Upper concentrating gel was 6% acrylamide. Samples for electrophoresis was prepared as follows: 1% 2-mercaptoethanol and 2% Ds-Na detergent were added to the medium (detergent: chlorophyll=20:1 (mg)) and incubated at room temperature for 30 min. Samples corresponding to 50µg protein were applied to each slot.

Thylakoid membrane proteins were analyzed according to Laemmi using a 10 to 25% (w/v) linear gradient polyacrilamide gel in the presence of SDS as described earlier (Guseynova et al., 2006). To each slot, 20-45 µl of samples (an equal Chl content) were applied. After electrophoresis, the gels were stained for 30 min (before boiling) with a solution of 0.04% Coomassie Brilliant Blue G-250 (France) prepared in 3.5% perchloric acid ($HClO_4$). The gels were scanned using an Ultroscan 2202 Densitometer (LKB, Sweden) with a 633 nm laser as the light source. If necessary gels were dried in a special device (Slab Gel Dryer – 2003, LKB, Sweden). A set of standard proteins consisting of bovine serum albumin (66 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), and lactalbumin (14.2 kDa) (sigma, USA) was used for the determination of the molecular masses of polypeptides.

## RESULTS AND DISCUSSION

The polypeptide patterns of the mesophyll (M) and bundle sheath (BS) thylakoids isolated from maize chloroplasts is shown in Fig.1. About 25 polypeptides ranged from 68 kDa to 10 kDa were observed using the gradient (10-25%) electrophoresis method. Laser densitogram of the gel is presented in Figure 2. Protein contents of photosynthetic membranes of mesophyll and bundle sheath chloroplasts were found to differ in both quantity and quality.

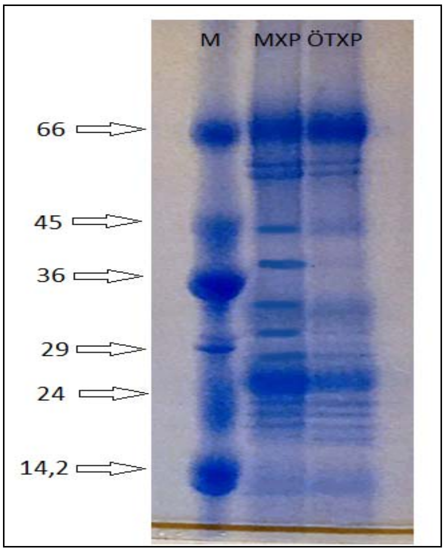



**Fig. 1.** Electrophoregram of thylakoid membranes of mesophyll and bundle sheath chloroplasts of maize on 10-25% PAAG in the presence of 0.1% DS-Na. MChl-thylakoids of mesophyll chloroplasts, BChl-thylakoids of bundle sheath chloroplasts, M-protein markers, kDa.

As seen in electrophoregram (Fig.1) and densitogram (Fig. 2) the protein composition of mesophyll thylakoids is similar to that of chloroplasts of typical higher plants ($C_3$ plants). Contrary to mesophyll thylakoids, some polypeptides lack in the protein content of bundle sheath thylakoids and amounts of others are reduced. As seen in the figures the amount of the PS I core apoprotein with molecular mass of 68 kDa is greater in bundle sheath thylakoids compared with mesophyll cell thylakoids. α and β subunits (with molecular masses of 55 kDa and 52 kDa) of CF1 domain of the ATPase complex are present in both subcellular fractions.

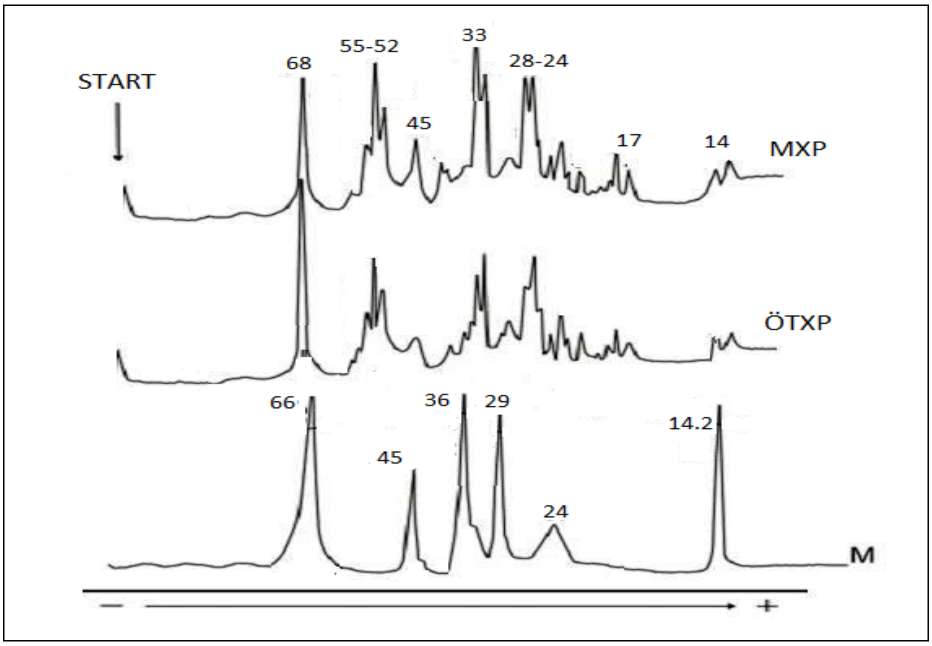



**Fig. 2.** Densitogram of thylakoid membrane proteins of mesophyll and bundle sheath chloroplasts of maize on 10-25% PAAG in the presence of 0.1% DS-Na. MChl-thylakoids of mesophyll chloroplasts, BChl-thylakoids of bundle sheath chloroplasts, M-protein markers, kDa.

However, the amount of α-subunit is smaller in thylakoids of BS cells. The protein of 45 kDa belonging to the PS II core antenna is more intensive in mesophyll thylakoids. Polypeptides (with molecular masses of 28-24 kDa) of the light-harvesting complex (LHC) of PSII are observed in both types of thylakoids, though their amounts are reduced in BS cells. Moreover, 33 kDa and 23 kDa proteins in the composition of oxygen-evolving complex (OEC) are observed in mesophyll cells and in relatively less amounts in thylakoids of BS cells. This confirms that chloroplasts of BS cells contain PS II complex, 33 kDa and 23 kDa polypeptides of oxygen-evolving complex. According to literature data immunoblot analysis revealed the existence of α-subunit in $CF_1$ domain of ATP-syntase complex, 33 kDa and 23 kDa proteins of the oxygen-evolving complex and polypeptides of LHCII and D1, D2 proteins in BS chloroplasts.

Thus, according to the obtained results, thylakoid membranes of mesophyll and bundle sheath chloroplasts in maize leaves have been found to differ in polypeptide contents.

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## Qarğıdalı Xloroplastlarının Mezofil və Örtüktopa Tilakoidlərinin Zülal Tərkibi

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Fotosintez edən oksigenli növlərin bir neçə, müxtəlif multisuvahiddən ibarət zülal komplekslərində təşkil olunmuş enerji çevirən aparatı tilakoid membranları ilə assosiasiya olunur. $C_4$ bitkilərin xarakteristik xüsusiyyəti fotosintetik yarpaq toxumalarının iki müxtəlif hüceyrə tipinin olmasıdır: mezofil (M) və örtük topa (ÖT) hüceyrələri. Bu tədqiqatda qarğıdalının (*Zea mays* L.) mezofil və örtük topa tilakoidlərinin polipeptid tərkibi analiz edilmişdir. Müəyyən edilmişdir ki, FSI-in nüvəsinə daxil olan apozülalının miqdarı (68 kDa) mezofillə müqayisədə örtüktopa tilakoidlərində daha çoxdur. ATP-sintaza kompleksinin $CF_1$ domeninin α və β - subvahidlərinə aid olan 55 kDa və 52 kDa molekul kütləli zülallar hər iki tip subhüceyrə fraksiyalarında vardır, lakin α subvahidinin miqdarı örtüktopa tilakoidlərində bir qədər azdır. FSII-nin nüvə antennasının molekul kütləsi 45 kDa olan zülalı mezofil tilakoidlərində daha intensivdir. İşıqtoplayıcı kompleks II-nin (LHC II) tərkibinə daxil olan (28-24 kDa) polipeptidlər hər iki tip tilakoidlərdə müşahidə edilir, lakin örtük topa hüceyrələrinin tilakoidlərində onların miqdarı reduksiya edilmişdir.

***Keywords:*** *$C_4$ plants, mesophyll, bundle sheath, chloroplasts, thylakoids, Zea mays*

## Белковый Состав Мезофильных и Обкладочных Тилакоидов Хлоропластов Кукурузы

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У видов кислородного фотосинтеза аппарат преобразования энергии, сформированный из нескольких белковых комплексов, содержащих различные мультисубъединицы, ассоциирован с мембранами тилакоидов. Характерной особенностью С4 растений является наличие у фотосинтетических тканей листа двух типов клеток: мезофильных (М) и обкладочных (О). В данном исследовании проведен анализ полипептидного состава мезофильных и обкладочных тканей тилакоидов кукурузы (Zea mays L.). Установлено, что по сравнению с мезофильными клетками, в клетках обкладки содержание апобелка (68 kDa), входящего в ядро ФС 1, намного выше. В обеих субклеточных фракциях присутствуют белки с молекулярной массой 55 kDa və 52 kDa, относящиеся к α və β субъединицам $CF_1$ домена АТФ-синтетазного комплекса, однако содержание α субъединицы в обкладочных тилакоидах несколько меньше. Белок ядерной антенны ФС 2 с молекулярной массой 45 kDa более интенсивен в мезофильных тилакоидах. Полипептиды (28-24 kDa), входящие в светособирающий комплекс II, наблюдаются в обоих тилакоидах, однако в обкладочных тилакоидах их число редуцировано.

***Ключевые слова:*** *$C_4$ растения, мезофилл, обкладка, хлоропласты, тилакоиды, кукуруза*